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RESEARCH ARTICLES

Vitamin D3 pada Penanda Stres Oksidatif dan Tekanan Darah pada Model Tikus UUO dari Penyakit Ginjal Kronis

Vitamin D3 on Oxidative Stress Markers and Blood Pressure in a UUO Rat Model of Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) is a major global health burden, with oxidative stress and hypertension contributing significantly to its progression. Although vitamin D3 is known for its pleiotropic effects, its influence on oxidative stress markers in obstructive nephropathy remains unclear. This study evaluated the effect of vitamin D3 supplementation on blood pressure, malondialdehyde (MDA) levels, and superoxide dismutase (SOD) activity in a rat model of CKD induced by Unilateral Ureteral Obstruction (UUO). In this experimental post-test-only control group study, 30 male white rats (Rattus norvegicus) were randomly assigned to five groups (n = 6 per group) following UUO induction: one control group (placebo) and four treatment groups receiving graded oral doses of vitamin D3 (36, 72, and 108 IU/rat) daily for 14 days. Blood pressure was measured using the tail-cuff method, and serum MDA levels and SOD activity were analyzed at the end of the treatment period. Vitamin D3 supplementation produced significant, dose-dependent improvements in all parameters (p-value < 0,01). Compared with controls, treated groups showed lower blood pressure, reduced MDA levels, and increased SOD activity. The highest dose (108 IU/rat) demonstrated the greatest reduction in oxidative stress and blood pressure. This study aimed to evaluate whether vitamin D3 supplementation ameliorates hypertension and oxidative stress in UUO-induced CKD in rats, supporting its potential as an adjunctive strategy to slow CKD progression. Further studies are required to confirm clinical applicability.

Keywords: *chronic kidney disease, oxidative stress, vitamin D3*

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Abstrak

Penyakit ginjal kronis (PGK) adalah beban kesehatan global yang utama, dengan stres oksidatif dan hipertensi berkontribusi secara signifikan terhadap perkembangannya. Meskipun vitamin D3 dikenal karena efek pleiotropiknya, pengaruhnya terhadap penanda stres oksidatif pada nefropati obstruktif masih belum jelas. Penelitian ini mengevaluasi efek suplementasi vitamin D3 pada tekanan darah, kadar malondialdehida (MDA), dan aktivitas superoksida dismutase (SOD) pada model PGK tikus yang diinduksi oleh Unilateral Ureteral Obstruction (UUO). Dalam studi kelompok kontrol eksperimental pasca-tes saja ini, 30 tikus putih jantan (Rattus norvegicus) secara acak ditugaskan ke lima kelompok (n = 6 per kelompok) setelah induksi UUO: satu kelompok kontrol (plasebo) dan empat kelompok perlakuan menerima dosis oral bertahap vitamin D3 (36, 72, dan 108 IU/tikus) setiap hari selama 14 hari. Tekanan darah diukur menggunakan metode tail-cuff, dan kadar MDA serum dan aktivitas SOD dianalisis pada akhir periode pengobatan. Suplementasi vitamin D3 menghasilkan peningkatan yang signifikan dan tergantung dosis di semua parameter (*p-value* < 0,01). Dibandingkan dengan kontrol, kelompok yang diobati menunjukkan tekanan darah yang lebih rendah, penurunan kadar MDA, dan peningkatan aktivitas SOD. Dosis tertinggi (108 IU/tikus) menunjukkan pengurangan terbesar dalam stres oksidatif dan tekanan darah. Temuan ini menunjukkan bahwa suplementasi vitamin D3 memperbaiki hipertensi dan stres oksidatif pada PGK yang diinduksi UUO pada tikus, mendukung potensinya sebagai strategi tambahan untuk memperlambat perkembangan PGK. Studi lebih lanjut diperlukan untuk mengonfirmasi penerapan klinis.

Kata Kunci: penyakit ginjal kronis, stres oksidatif, vitamin D3

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Highlight:

- Vitamin D3 supplementation led to significant, dose-dependent improvements in reducing blood pressure and malondialdehyde (MDA) levels, while simultaneously increasing superoxide dismutase (SOD) antioxidant activity.
- The study found that the highest dose tested (108 IU/rat) was the most effective at reducing oxidative stress and lowering blood pressure in the rat model of chronic kidney disease.
- These findings suggest that Vitamin D3 could serve as a valuable adjunctive strategy to help slow the progression of chronic kidney disease by managing hypertension and oxidative stress.

INTRODUCTION

Chronic kidney disease (CKD) is a slowly worsening disorder that directly afflicts more than 800 million individuals worldwide, and disproportionately in low-middle income countries (Kovesdy, 2022). The prevalence in Indonesia is approximately 0,5% (Hustrini, 2023) with affected individuals at a comparably young mean age of 44,3 years. The pathophysiology of CKD is multifactorial and hypertension, oxidative stress (OS), and inflammation are shown to be deleterious players that act synergistically during the progression of renal disease and

cardiovascular complications (Daenen et al., 2019). A frequent comorbidity, present in up to 80% of patients, is a decreased level of 25-hydroxyvitamin D3 (25[OH]D), the key indicator of vitamin D status (Gois et al., 2018). This deficiency is not only a consequence of declining kidney function, but may play an active role in the development and progression of CVD.

It is now appreciated that vitamin D3 has pleiotropic properties in addition to its role in regulation of minerals. Its biologically active form, 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$), exerts its action by binding to the vitamin D receptor (VDR) that is expressed in various tissues such as the vascular endothelium and kidney. Vitamin D3 may affect important pathways in the pathophysiology of CKD, via activation of the VDR. It induces modulation of the renin-angiotensin-aldosterone system (RAAS), which plays a pivotal role in hypertension and renal fibrosis (Mehta and Agarwal, 2017; Verma et al., 2021). It also has antioxidant effects that may be achieved through the upregulation of nuclear factor erythroid 2-related factor 2 (Nrf2) -directed expression of antioxidant enzymes, including SOD and GPx, along with the inhibition of ROS generation and lipid peroxidation markers such as MDA (Cojic et al., 2021; Omar et al., 2022; Kamel et al., 2022). This dual effect on RAAS and redox state suggests that vitamin D3 may represent a valuable treatment for reducing hypertension as well as oxidative insult in CKD.

The rodent model of UUO has long been a classic experimental paradigm to investigate the pathogenesis of obstructive nephropathy, which is one of the most common causes for CKD. UUO initiates a cascade of pathological events, such as increase in intratubular pressure, ischemia, inflammation and eventually fibrosis that collectively mimics certain aspects of progressive kidney damage (Klimova et al., 2023; Martínez et al., 2023). Importantly, systemic effects of the UUO model has been reported. The first renal hit induces the RAAS, which results in vasoconstriction and sodium retention and circulating systemic blood pressure can increase (Verma et al., 2021). Simultaneously, the marked intrarenal OS increase also feeds into the systemic generation and circulation of ROS and lipid peroxidation-derived products (e.g., MDA), at the expense of endogenous antioxidant capacity (the lower SOD activity) (Morales and Munné-Bosch, 2019). Thus, the UUO model serves as a reproducible and controlled setting to study interventions aimed at modulating renal and systemic pathophysiology.

The tight relationship between vitamin D3 deficit and concurrence of adverse events in CKD patients including the UUO model has been well defined but the impact of standardized vitamin D3 treatment on SBP and serum oxidative stress markers in UUO animals has not been clearly identified. Previous studies have been mixed, especially related to effects on blood pressure (Sanuade et al., 2023), and all have not used graded doses of the drug that are clinically relevant nor a validated outcome measure. A distinct void exists with regard to whether vitamin D3 supplementation under experimental conditions could simultaneously attenuate hypertension, lower the levels of lipid peroxidation (MDA) and increase activity of antioxidant enzyme (SOD) in obstructed nephropathy.

This study hypothesizes that supplementation with vitamin D3 in rats dose-dependently reduces blood pressure (BP) level and malondialdehyde (MDA) content in the systemic circulation and increased SOD activity in a model of CKD induced by UUO. The purpose of this study was to explore the effects of different doses of vitamin D3 on blood pressure, serum MDA, and serum SOD in male white rats (Rattus norvegicus) after unilateral ureteral obstruction.

METHODS

The study was conducted using a true-experimental post-test-only control group design investigating the effects of vitamin D3 on UUO animal models with male white rats as subjects (Agnesia et al., 2023; Liberty, 2024). This research was approved by the Research Ethics Commission of Faculty of Medicine UNS/RS dr Moewardi Surakarta with Ethical Clearance number:2.214/XII/HREC/2023. Thirty healthy male Wistar rats aged 8 weeks and weighing 150-200 g were obtained from the Faculty of Veterinary Medicine, Gadjah Mada University. According to material equation calculation for animal experiment Festing et al. (2002), Thirty male Wistar rats were divided into five groups (n=6 per group). Animals were acclimatized in conventional polypropylene cages for 14 days under controlled conditions (22±2°C, 50-60% relative humidity and 12-hour light/dark cycle) at Animal Husbandry PAU UGM Yogyakarta with ad libitum access to standard rodent chow (BR I, Indonesia) and filtered water. The five experimental groups consisted of sham operation with daily vehicle (0,2 mL olive oil) group as control (Group 1), UUO induction with daily vehicle group as non-treatment group (Group 2), and UUO induction with daily oral cholecalciferol [vitamin D3] at the dose of 36 IU/rat (low-dose; Group 3), 72 IU/rat (medium-dose; Group 4) or 108 IU/rat group (high-dose; Group 5). For UUO, the left ureter was doubly ligated and divided between ligatures via a left flank incision after induction with ketamine-xylazine anesthesia (80 mg/kg; 10 mg/kg IP) to produce a stable occlusion, whereas sham-operated animals received only ureter exposure without ligation. Post-surgical treatment consisted of heat pad recovery, enrofloxacin (10 mg/kg IM) and buprenorphine (0,05 mg/kg SC) for 48 hours.

The vitamin D3 treatment began 24 h after the surgery application of CS implant and was applied daily for 28 days by oral gavage, in doses corresponding to the animal's body weight. At study termination (Day 27), the animals' systolic and diastolic blood pressure was non-invasively measured using a CODA tail-cuff system (Kent Scientific, U.S.A.) after a 3-day acclimatization period, with 15 consecutive cycles taken per rat and with the first five cycles discarded; Mean Arterial Pressure (MAP) was calculated as Diastolic BP +1/3(Systolic BP – Diastolic BP). In the day 28 of this experiment, after a fasting period of 8-10 hours, animals were anaesthetised deeply and blood samples of approximately 3 mL were collected by orbital sinus puncture; they then underwent centrifugation at 3000 rpm for 15 minutes at 4°C and serum was kept frozen to -80°C until analysis.

The left kidneys were examined visually to ensure that UUO was induced successfully through inspection for hydronephrosis, and animals were next sacrificed by cervical dislocation under deep anesthesia. Serum malondialdehyde (MDA) and superoxide dismutase (SOD) levels were quantified using commercial ELISA kits (FineTest, Lot Nos. ER0586 and ER0214 respectively), in strict accordance with the manufacturer's directions, with all the samples examined in duplicate. Statistical analysis was performed with the IBM SPSS Statistics Version 25.0. Descriptive statistics (mean ± standard deviation) for all variables were determined. Homogeneity of variances was tested with Levene's and normality using Shapiro-Wilk test. Parametric data (blood pressure and SOD levels) were analyzed using one-way ANOVA continued with post hoc test of Tukey's HSD, non-parametric data (MDA levels) were analyzed by Kruskal Wallis followed by Dunn test using Bonferroni correction and are significant if p-value < 0,05 (Darma, 2021; Hardani et al., 2020).

RESULTS AND DISCUSSIONS

Following UUO induction, all animals in groups P2-P5 survived the surgical procedure and the 28-day treatment period. At necropsy, successful UUO was visually confirmed in all animals from groups P2-P5 by the presence of marked left hydronephrosis, which was absent in the sham-operated group (P1).

Body weight of rats

Table 1 presents the initial and final body weights of all groups. At baseline, there were no statistically significant differences in mean body weight among the groups (p = 0,387), confirming successful randomization. After 28 days, a one-way ANOVA revealed significant differences in final mean body weight across groups (p < 0,001). The sham control group (P1) showed the highest weight gain, while the UUO control group (P2) showed the lowest. Vitamin D3-treated groups exhibited a dose-dependent increase in final body weight compared to the untreated UUO control, with the high-dose group (P5) approaching the weight of the sham control.

Table 1. Body weight changes of rats

Group	Initial Body Weight (g)	Final Body Weight (g)	Mean Weight Gain (g)
P1 (Sham Control)	185,25 ± 3,01	234,25 ± 3,06	49,00
P2 (UUO Control)	188,00 ± 2,34	210,38 ± 2,50	22,38
P3 (UUO + Vit D3 36 IU)	186,75 ± 2,67	216,88 ± 3,27	30,13
P4 (UUO + Vit D3 72 IU)	185,62 ± 3,29	223,88 ± 4,12	38,26
P5 (UUO + Vit D3 108 IU)	186,12 ± 3,31	224,12 ± 3,44	38,00
p-value	0,387a	<0,001a,*	

Note: Data are presented as mean ± standard deviation. P1: sham-operated + vehicle; P2: UUO + vehicle; P3: UUO + vitamin D3 36 IU/rat; P4: UUO + vitamin D3 72 IU/rat; P5: UUO + vitamin D3 108 IU/rat. a = one-way ANOVA test; * = statistically significant (p -value < 0,05).

Effects of vitamin D3 on blood pressure, MDA, and SOD

The effects of graded vitamin D3 supplementation on blood pressure, serum MDA levels, and serum SOD activity are summarized in Table 2. UUO induction in the control group (P2) resulted in severe hypertension, a marked elevation in the lipid peroxidation marker MDA, and a profound depletion of the antioxidant enzyme SOD compared to the sham control (P1), confirming the successful establishment of the disease model. Treatment with vitamin D3 for 28 days resulted in significant, dose-dependent improvements across all parameters. One-way ANOVA revealed statistically significant differences among all groups for mean arterial pressure (MAP), MDA, and SOD (p < 0,001 for all).

Table 2. Blood Pressure, MDA, and SOD levels across study groups

Group	Mean Arterial Pressure (mmHg)	MDA (nmol/mL)	SOD (ng/mL)
P1 (Sham Control)	83,50 ± 1,77	1,33 ± 0,18	85,11 ± 3,81
P2 (UUO Control)	215,50 ± 3,93	10,71 ± 0,36	24,27 ± 3,60
P3 (UUO + Vit D3 36 IU)	138,50 ± 7,98	4,97 ± 0,23	58,46 ± 4,14
P4 (UUO + Vit D3 72 IU)	105,50 ± 3,82	2,94 ± 0,32	64,52 ± 4,27
P5 (UUO + Vit D3 108 IU)	97,13 ± 4,02	2,12 ± 0,15	76,84 ± 4,29

Group	Mean Arterial Pressure (mmHg)	MDA (nmol/mL)	SOD (ng/mL)
p-value	<0,001*	<0,001*	<0,001*

Note: Data are presented as mean ± standard deviation. MAP was calculated from systolic and diastolic tail-cuff measurements. MDA and SOD were measured in serum via ELISA. P1: sham-operated + vehicle; P2: UUO + vehicle; P3: UUO + vitamin D3 36 IU/rat; P4: UUO + vitamin D3 72 IU/rat; P5: UUO + vitamin D3 108 IU/rat. * = statistically significant (p-value < 0,05) one-way ANOVA test.

As shown in Table 2 and detailed in Table 3, all doses of vitamin D3 significantly lowered blood pressure and MDA, and increased SOD compared to the untreated UUO control group (P2). The effects were dose-dependent, with the high-dose group (P5, 108 IU/rat) demonstrating the greatest improvement across all outcomes. P5 exhibited significantly lower blood pressure and MDA, and significantly higher SOD activity than both the medium-dose (P4) and low-dose (P3) groups. Notably, blood pressure and MDA levels in the high-dose group (P5) approached, but did not fully normalize to, the levels observed in the sham control group (P1).

Table 3. Pairwise comparisons and effect sizes for blood pressure, MDA, and SOD

Comparison	Blood Pressure (MAP)		MDA		SOD	
	Mean Diff. [95% CI]	*p-value*	Mean Diff. [95% CI]	*p-value*	Mean Diff. [95% CI]	*p-value*
P2 vs. P1	132,00 [126,28, 137,72]	<0,001*	9,38 [9,04, 9,72]	<0,001*	-60,84 [-65,44, -56,24]	<0,001*
P3 vs. P2	-77,00 [-82,72, -71,28]	<0,001*	-5,74 [-6,08, -5,40]	<0,001*	34,19 [29,59, 38,79]	<0,001*
P4 vs. P2	-110,00 [-115,72,-104,28]	<0,001*	-7,77 [-8,11, -7,43]	<0,001*	40,25 [35,65, 44,85]	<0,001*
P5 vs. P2	-118,37 [-124,09, -112,65]	<0,001*	-8,59 [-8,93, -8,25]	<0,001*	52,57 [47,97, 57,17]	<0,001*
P5 vs. P3	-41,37 [-47,09, -35,65]	<0,001*	-2,85 [-3,19, -2,51]	<0,001*	18,38 [13,78, 22,98]	<0,001*
P5 vs. P4	-8,37 [-14,09, -2,65]	0,002*	-0,82 [-1,16, -0,48]	<0,001*	12,32 [7,72, 16,92]	<0,001*

Note: Data are presented as mean difference [95% Confidence Interval]. Post-hoc comparisons were performed using Tukey's HSD test for parametric data (Blood Pressure, SOD) and Dunn's test with Bonferroni correction for non-parametric data (MDA). P1: sham-operated + vehicle; P2: UUO + vehicle; P3: UUO + vitamin D3 36 IU/rat; P4: UUO + vitamin D3 72 IU/rat; P5: UUO + vitamin D3 108 IU/rat. * = statistically significant after correction (p-value < 0,05)

In the present investigation, 28-day supplementation with an active vitamin D3 (cholecalciferol) abrogated UUO-induced hypertension and improved systemic oxidative stress in a dose-dependent manner as evident from reduced serum MDA concentrations and increased serum SOD activity. Although these results are consistent with our hypothesis and with an expanding literature, when interpreting these findings one should consider alternative interpretations, caveats of the study and context in a larger mechanistic framework.

Vitamin D3 and blood pressure

The dose-dependent fall in MAP (UUO rats; vitamin D3 treatment: 215,5 mmHg in P2→97,1 mmHg in P5) is amazing and the potent antihypertensive activity in this model has been confirmed. Data from Lu et al. (2021), are consistent with the established findings that role of vitamin D in blood pressure but the size of the effect adds caution to confounding issues that need address. The very high blood pressure observed in the untreated UUO group (P2), which is consistent with severe obstructive nephropathy and renin-angiotensin aldosterone system (RAAS) activation, may have been further exaggerated by stress from the tail-b cuff measurement protocol. Even though the rats had been acclimatized for 3 days, this device did not confirm using telemetry, which is a limitation. Moreover, the dose-dependent elevation in final body weight exhibited by treated groups (Table 1) represents a potential confounding factor as better nutritional status and increasing body weight might independently modify cardiovascular parameters. Nevertheless, the reduction in blood pressure closest to sham levels in the high-dose group (P5) who did not recover to sham weight suggests a directly pharmacological effect rather than solely metabolic (Chakhtoura and Alam, 2025; Sheikh et al., 2020).

The antihypertensive action is presumably a composite one. Vitamin D3 is a negative endocrine regulator of the RAAS. By competing with renin inhibitor, it inhibits the expression of renin by binding to VDR in the juxtaglomerular apparatus and mediated inhibition of angiotensin II and aldosterone (Jensen et al., 2023; Verma et al., 2021). In the UUO model where RAAS activation is a major mechanism driving hypertension and fibrosis, this pathway provides an attractive mechanistic basis for our observations. Further, vitamin D3 stimulates Enos activity leading to vasodilatation and endothelial function (Arendshorst et al., 2024; Fountain et al., 2023).

Effect of vitamin D3 on oxidative stress (MDA and SOD)

The UUO model is typified by severe oxidative stress, partly through mitochondrial dysfunction, infiltrating immune cells and NADPH oxidase induction (Ding et al., 2020). We observed that serum MDA (a marker of lipid peroxidation) was significantly increased by UUO, while SOD (one of the preeminent endogenous antioxidant enzymes) was significantly reduced. The dose-dependent restoration of these abnormalities through vitamin D3 strongly suggests an in vivo antioxidation action.

The decrease in MDA indicates that D 3 protects cell membrane against oxidative damage. This is in agreement with findings that vitamin D3 can decrease the expression and activity of NADPH oxidase (Nox2, Nox4) subunits, and increase uncoupling proteins (UCPs), inhibiting mitochondrial production from ROS inside cells (Cojic et al., 2021; Wee et al., 2021). One caveat is that only one marker of oxidative stress is used in the present study. Although MDA is a commonly used and accepted biomarker, the measurement of this product may be affected by sample acquisition and diet, and does not represent the full complexity in oxidative damage (Cordiano et al., 2023). Further studies should incorporate more specific markers of oxidative damage (ie F2-isoprostanes for lipid peroxidation, or 8-hydroxy-2'-deoxyguanosine [8-OHdG] for DNA damage) in order to give a stronger indication.

The elevation of SOD in serum level is also significant. SOD catalyzes the dismutation of superoxide radicals, which are released as the first ROS in mitochondria and by NADPH oxidase. Vitamin D3 increases the kidney's and systemic circulation's ability to mitigate superoxide through repletion of SOD activity, thus ameliorating the

cycle of ROS-mediated injury. The VDR appears to be involved in this effect due to its translocation to the nucleus and ability, upon binding of a ligand, upregulate the transcription of antioxidant response elements, perhaps through interactions with the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Anwar et al., 2025; Backston et al., 2025).

Study limitations

A number of important limitations of this study need to be discussed. First, the post-test-only design which is valid does not allow examination of within-subject changes over time. Second, there were no renal functional data (serum creatinine and blood urea nitrogen, proteinuria). With the decrease in oxidative stress, we assume better kidney health, but I do not prove that it was preserved renal function from vitamin D3. Third, we quantified systemic/circulating markers of oxidative stress. Although these data are informative, they do not necessarily represent the intrarenal redox environment. Finally, a single blood pressure measurement method was not validated telemetrically, rendering the results not immune to artifacts due to stress. Lastly, 28 days is an appropriate exposure time to detect biochemical effects but too short for evaluation of long-term survival or consequences on renal fibrosis.

CONCLUSIONS

The findings showed that 28-days of vitamin D3 (cholecalciferol) treatment in a UUO rat model significantly and dose-dependently decreases mean arterial pressure (MAP), serum malondialdehyde levels, and increases serum superoxide dismutase activity, indicating that vitamin D3 has potential beneficial effects on hypertension and oxidative stress as major contributors to the progression of CKD. Nonetheless, these preclinical hypothesis generating results need to be put into perspective, since there are several limitations in the CKD model i.e., no functional CKD validation (e.g. serum creatinine, proteinuria, histology), no measurement of vitamin D status (25[OH]D) and safety markers like calcium or parathyroid hormone. Thus, although the data demonstrate promise for vitamin D3 as an adjunctive therapy in CKD, they cannot be directly extrapolated to clinical practice. Second, the present study had several limitations that should be addressed in future research, including a more comprehensive CKD phenotyping with functional and histologic endpoints (e.g., amount of proteinuria and degree of histological damage) to further explore the infiltrates observed here; studying inflammatory cytokines (e.g., IL-6) and RAAS activity directly in renal tissue; comparing cholecalciferol versus calcitriol treatment groups; including females for potential sex differences analysis; analyzing telemetric blood pressure monitoring to exclude stress-related artifacts. The validity of such promising associations and clinical value of vitamin D3 in chronic kidney disease both need to be addressed further by well-designed mechanistic as well as pharmacological studies.

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